Title
Optical Imaging of Early Dental Caries in Deciduous Teeth with Near-IR light at 1310nm

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Optical Imaging of Early Dental Caries in Deciduous Teeth with Near-IR light at 1310nm

by

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THESIS

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Abstract

Dental caries is a widespread disease that affects many children. Through a concerted effort by the parents, dentist, and child, the decay process can be arrested/reversed by non-surgical means via fluoride therapy, antimicrobial rinses, dietary changes, or low-intensity laser irradiation. However, success of these preventative measures depends to a great extent on the early detection of the decay process. Traditionally, dentists have utilized tactile and visual techniques to detect caries, but unfortunately these methods have a low sensitivity. Conventional radiography (x-rays) is adequate for large, cavitated lesions; however, it does not have sufficient sensitivity for the detection of early, non-cavitated caries, root surface caries, or secondary caries. Moreover, due to the distinct morphology of the primary dentition, including thin enamel, proportionally thinner dentin, and large pulp chambers, the progression of caries is intensified. Therefore, new methods for early caries detection are necessary. Recently, new technology has been approved for the diagnosis of decay, e.g. the DIAGNOdent and QLF, but these devices detect caries in its late stages when surgical intervention is usually necessary. Moreover, these devices do not provide depth-resolved images of the caries and therefore, cannot be used to assess the effectiveness of properly implemented preventative measures.

Polarization-sensitive optical coherence tomography (PS-OCT) is a non-invasive imaging technique which utilizes near-infrared (NIR) light to produce depth-resolved images of dental enamel and has the potential to monitor early enamel caries. The scattering of light in the sound enamel and dentin is

sufficiently strong in the visible range to obscure light transmission through the tooth. However, in the near-IR at 1310nm, enamel is highly transparent. The tissue contrast with devices utilizing near-IR light arises from changes in tissue scattering as opposed to conventional radiography, which is based on variations in tissue density. Therefore, this method can be more sensitive than x-rays in detecting early caries lesions [25].

The potential of NIR imaging of early caries lesions in primary teeth has not been investigated. These methods do not require exposure to ionizing radiation, deciduous teeth are smaller and more accessible, and the dentist can avoid the difficult procedure of placing x-ray films in uncooperative children. However, primary teeth differ in composition and structure from the permanent dentition and therefore, their optical properties are expected to be different.

The overall objective of this research is to develop non-invasive optical devices for the detection and diagnosis of early dental caries in deciduous teeth. The objectives of this study will be achieved through the following specific aims:

AIM # 1: *To test the hypothesis that the scattering coefficient of sound deciduous enamel is orders of magnitude less at 1310 nm than in the visible range.*

AIM # 2: *To test the hypothesis that trans-illumination with NIR can discriminate carious enamel from sound enamel with high contrast.*

AIM # 3: *To test the hypothesis that PS-OCT can image and quantify occlusal caries lesions.*

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Introduction

Background

Dental caries is the most common, chronic disease of childhood. It has been estimated that this disease is 5 times more prevalent than asthma and seven times more prevalent than hay fever. Furthermore, the disease is neither self-limiting nor amenable to treatment with any single therapy like antibiotics[1]. According to a study performed by Mouradian *et al.*, by mid childhood, more than 50% of children have detectable caries, and by late adolescence about 80% have acquired this preventable infectious disease[2]. It has been reported that dental care is the most prevalent unmet health need among American children and that 51 million school hours per year are lost in the United States because of dental related illness[1]. In fact, if left untreated dental tooth decay may cause a failure to thrive in young children and provides a reservoir for infection that aides in the formation of abscesses, cellulitis and septicemia. Malocclusion has also been linked to premature loss of deciduous teeth due to advanced caries disease. Furthermore, on a long term scale, decayed primary teeth have been shown to be a reliable predictor of tooth decay in the permanent dentition[2]. Therefore, the establishment of preventative measures is imperative in the control of this disease. Berg[3] identified two important tasks that the practitioner should recognize. The first is identification of the population that is most at risk for developing caries, i.e. children of low socioeconomic backgrounds and special needs patients. The second is to manage the caries process by preventing the disease from manifesting itself in the form of a cavity in the first place. Weinstein[4] mentions that the epidemic of caries cannot be solved solely by providing treatment. Traditional restorative dental treatments do not stop the caries process. In fact, once a tooth is cut to receive a restoration, it is at risk for recurrent decay and the subsequent placing of a larger restoration if the unfavorable oral environment that caused the cavity persists.

The caries process, however, can be arrested in its early stages [1-7]. The time required for remineralization to replace the lost minerals during demineralization is determined by the age of the plaque, the nature of the carbohydrate consumed, and the presence or absence of fluoride[1]. With recent developments in fluoride formulations, antimicrobial therapy, and early preventative measures, the caries process can be stopped and even reversed. Therefore, prevention via health counseling and guidance should be the cornerstone of the dental profession if it intends on controlling this communicable disease. Many other preventative measures have been proposed. Most notably, the American Academy of Pediatric Dentistry has emphasized that all infants should have their first dental visit by the time they are 12 months. In terms of caries prevention, this first visit plays a vital role in the prevention of tooth decay in young children by emphasizing important concepts such as diet, fluoride use, and oral hygiene [2,4,5,6]. Nevertheless, dental caries continues to be the most prevalent disease of childhood even though great strides have been made in improving children’s oral health.

Despite the wide spread contagion of the caries disease, attempts have been made in detecting lesions early enough so that they may be remineralized and arrested. New dietary and restorative products have been formulated in the hope of halting the widespread transmission of this disease. Products such as xylitol have shown favorable results in stopping the caries process. Xylitol is a sugar alcohol with sweetness equal to that of sucrose, but with 40% fewer calories[8]. In a study performed by Ly *et al.*, xylitol has been shown to be non-cariogenic. Furthermore, it has been demonstrated that xylitol has a caries protective effect by reducing plaque and salivary Mutans Streptococci (MS) levels and by reducing the level of lactic acid produced by these bacteria[8]. Microorganisms do not readily metabolize xylitol into energy sources, and its consumption has a minimal effect on plaque pH. Furthermore, it has been shown that xylitol inhibits the transfer of bacteria from person to person by altering the way the bacteria stick to surfaces[9]. Xylitol, therefore, has great potential as an adjunct in a dentist's armamentarium of tools for caries prevention.

Another preventative tool that has been investigated extensively is the use of fluoride in the dental practice. Fluoride has a profound effect on the remineralization process. In fact, it is a well-known fact that naturally occurring fluoride in the water supply leads to a decreased rate of dental caries in the populations that consume it [1]. In an effort to extend the systemic benefit of fluoride to populations in which water fluoridation was not readily available led to the development of various forms of fluoride formulations, including dentifrices, varnishes, foams, etc. with success rates ranging from 20% to 38% in the overall reduction of dental caries[7]. Furthermore, it has been shown that the greatest benefit from fluoride use is via topical application. The mechanism by which this occurs is explained by ten Cate *et al.* [10]. Recent observations indicate that demineralization is inhibited due to fluoride absorption from solution onto tooth apatite crystals forming fluorapatite. The resulting fluorapatite crystal has a very low solubility and the tooth enamel is more resistant to mineral dissolution at lower pH levels. Other benefits of fluoride are its antimicrobial effects on oral flora. The method by which this activity occurs lies in the ability of fluoride to inhibit enolase activity in bacteria. The enzyme enolase is a key enzyme in the glycolysis of glucose and its biotransformation into lactic acid. Therefore, inhibition of this enzyme reduces the amount of acid that bacterial species produce, hence allowing remineralization of early lesions.

Etiology of Dental Caries

Caries is a ubiquitous and multifacted disease that afflicts a great portion of the population. Although many individuals do not manifest the severe form of the disease, i.e. cavities, many are at risk due to the extent of demineralization that occurs throughout their mouth. To form a cavity, three important components must be in place, notably, the bacteria, the host, and a substrate (Figure 1). This model put forth by Fitzgerald and Keyes [11], describes the key determinants in the caries process. According to this model, a substrate in the form of a fermentable carbohydrate is needed for the bacteria to produce organic acids, which in turn initiates the demineralization of dental enamel. According to Anderson *et al.* [12], all three elements in this model are required for disease progression. Removal of any one element ostensibly leads to the interception of the disease process. The intricacy of the individual elements, however, puts forth an enormous challenge in thoroughly understanding the caries process. For example, various bacterial species live in symbiosis in the biofilm of teeth. This cohabitation, however, is not clear because many strains of bacteria have been identified and their pathogenesis in the decay process is not yet fully understood.

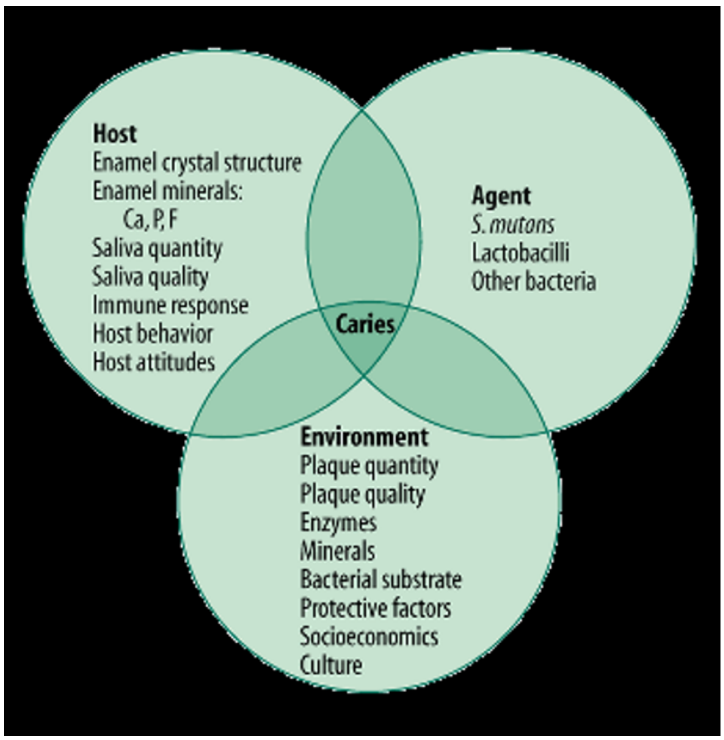


Figure 1. Multifactorial Model of Dental Caries

Studies on Mutans streptococci have revealed this group of bacteria as the most important contributor to dental decay [13], but other species, e.g. Lactobacilli and Veillonella sp., have also been identified as odontopathogens.

Moreover, the substrates metabolized and the pathways by which bacteria metabolize fermentable carbohydrates to form acid is an area of active research. It is known that cariogenic bacteria transform simple sugar molecules, e.g. glucose, fructose, and sucrose, into long chain organic acids via a glycolytic pathway. The acid by-product in turn diffuses into the tooth and dissolves the mineral, hence initiating the caries process. The critical pH for the dissolution of mineral in enamel is 5.5. The outer surface of enamel is far more resistant to demineralization by acid compared to the deeper portion of enamel[1]. Once the decay reaches the dentin, however, mineral loss can occur at a considerably higher pH. The continuation of this process results in the formation of an incipient, subsurface "white-spot" lesion. Unless this lesion is arrested and reversed, the lesion will continue to enlarge, with the eventual collapse of the thin surface layer and the formation of the cavitated lesion.

The host, the third link in the caries model, is also a key determinant in the caries process. Saliva produced by individuals performs important roles in the oral cavity. It serves as a buffer that maintains the pH at a physiologic level during episodes of acid attack by bacteria and regulates the oral flora [14]. Moreover, individuals with decreased levels of saliva due to medications, radiation, or disease have a strong, positive correlation with their level of dental decay. Similarly, individuals with poor oral hygiene, inadequate diet, or genetically predisposed individuals are at high risk for severe manifestations of the caries process[1]. In a recent paper by Crall *et al.* [6], it is noted that no single modality, such as fluoride or sealants, or alteration of the composition of bacteria in dental plaque should be expected to completely eliminate caries as a disease. Therefore, prevention and early detection is pivotal in preventing the infection from progressing.

Morphology of the Primary Dentition

The components of the primary dentition include 4 central incisors, 4 lateral incisors, 4 canines, and 8 molars for a total of 20 deciduous teeth. The first deciduous teeth to erupt into the oral cavity are the mandibular central incisors. These teeth usually erupt at 6 months of age and are usually followed by the maxillary central incisors. The primary dentition is considered complete by age 3 with eruption of the 2^{nd} primary molars. The primary dentition remains in the mouth until age 6, after which the mixed dentition begins.

The primary dentition has important functions. Notably, the primary teeth maintain space for permanent successors, they stimulate growth of the jaws through mastication, they facilitate correct mastication of food, assist in proper speech development, and are important for overall esthetics.

There are important morphologic differences between primary and permanent teeth (Figure 2 and 3). One of the differences includes the size difference of deciduous teeth and succedaneous teeth, with the deciduous teeth tending to be smaller than the permanent teeth. Moreover, primary teeth have markedly constricted necks compared to the permanent teeth and have contact areas rather than contact points as in permanent teeth. This latter point is important in plaque retention in primary teeth due to the broad contact area that leads to sustained levels of acidic pH for prolonged periods of time that in turn may lead to a higher prevalence of interproximal decay in the primary dentition. Furthermore, the enamel on primary teeth is much thinner and has a more consistent depth than in permanent teeth. It is estimated that the thickness of enamel in primary teeth is approximately 1 mm throughout the crown. The enamel rods at the cervix of primary teeth slope in an occlusal or incisal direction, rather than being gingivally oriented as in the permanent dentition. Moreover, there is comparatively less tooth structure protecting the pulp in primary teeth, placing the primary dentition at greater risk for caries progression. The deciduous teeth are also much lighter in color compared to their permanent counterparts. This may be due to the density of the enamel in primary teeth and their higher water content.

The progress of caries is intensified in the primary dentition because the enamel is much thinner, allowing the decay to progress more rapidly through the enamel layer; the dentin is proportionally thinner, allowing caries to reach the pulp much more rapidly; and the pulp is relatively larger and the pulp horns are more prominent, causing a greater risk of pulpal exposure when restoring decayed primary teeth.

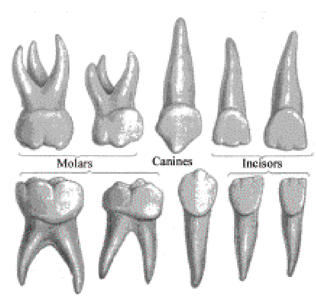


Figure 2 - Primary Teeth

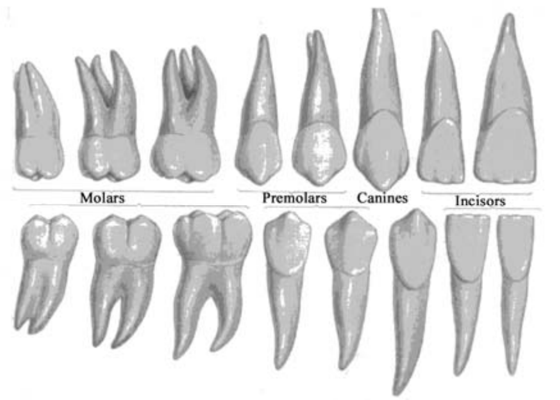


Figure 3 - Permanent Teeth

Early Detection of Caries Lesions

According to McDonald[1], 84% of children who were caries free in the primary dentition remained caries free in the mixed dentition. Moreover, children with pit and fissure caries in the primary dentition were more likely to develop smooth-surface caries of primary teeth than the caries-free children. 57% of the children with proximal lesions in primary molars developed additional primary molar proximal lesions in the mixed dentition. Furthermore, the primary teeth are susceptible to caries formation during the first 2 years after eruption because enamel calcification is incomplete at the time of eruption of the teeth and an additional period of approximately 2 years is necessary for the calcification process to be completed[1]. Managing the disease process in the primary dentition, therefore, is a formidable task despite advances in prevention. Clearly, alternative detection methods are necessary in order to detect early lesions that can be remineralized with early non-surgical methods including fluoride therapy, antimicrobial rinses, dietary changes, and low-intensity laser irradiation.

Traditionally, dentists have relied on visual examination of suspicious lesions on pits/fissures and smooth surfaces, radiographic examination of interproximal areas, and the use of a sharp explorer to assess the degree of resilience of a lesion. However, such diagnostic methods have a low sensitivity (~0.30), implying that only 20-48% of the occlusal caries present is actually found; specificity typically exceeds 0.95 [15]. Furthermore, radiographic detection of interproximal lesions also has poor sensitivity (~0.59) and high specificity, and as much as 25% of the interproximal areas of bitewing radiographs are unresolved due to the overlap with healthy tooth structure on adjacent teeth. Moreover, lesions detected on radiographs typically have progressed beyond the point that conservative therapy can be rendered and surgical intervention, in many cases, is the only option [3]. The principal limitation of bitewing radiographs for early caries detection is that they cannot by used to detect early occlusal lesions because of the convoluted topography of the crown[15-16]. Based on these inherent limitations of our current methods of detection and diagnosis, new, alternative methods have been developed and approved for clinical use.

An instrument designed to facilitate the detection of dental decay, DIAGNOdent, was developed for the detection and quantification of dental caries of occlusal and smooth surfaces. The device consists of a diode laser and a fiber optic probe designed to detect the near-IR fluorescence from porphyrins—a bacterial by-product. The principal limitation of this device is that it detects lesions in the later stage of development and has a poor sensitivity (~0.40) for lesions confined to enamel [1,3,15]. Another technique for early caries detection is based on quantitative laser fluorescence (QLF). With this method, a relationship is established between the loss of fluorescence and the extent of enamel demineralization. QLF is capable of early caries detection and is useful in monitoring the progression or regression of lesions. The major shortcoming with this method, however, is that it is confined for use on smooth surface lesions and cannot be readily applied to occlusal and interproximal caries lesions (which constitute the majority of lesions) [3,15]. In summary, fluorescence methods cannot provide depth resolved images of demineralization or lesion severity. According to McDonald [1], caries detection is entering a new era with new technologies capable of detecting lesions at an earlier stage of development and quantifying the impact of non-invasive professional fluoride treatments and other preventative techniques.

Polarization Sensitive-Optical Coherence Tomography (PS-OCT)

PS-OCT is a non-invasive imaging technique than can utilize near-infrared (NIR) light to produce depth-resolved images of dental enamel and has the potential to monitor early enamel caries [17-20]. PS-OCT has been successfully used to acquire images of both artificial and natural caries lesions, assess their severity in depth, asses the remineralization of such lesions and determine the efficacy of chemical agents in inhibiting demineralization. Polarization-sensitive depth-resolved reflectivity measurements can provide a measure of the severity of natural and artificial caries lesions on smooth surfaces and in the occlusal pits and fissures. The high reflectivity at the tooth surface produces a very strong reflection that can interfere with the measurement of early demineralization that is located on that surface. The magnitude of this strong reflection can be reduced using polarized light. If the incident illuminating light is polarized, the carious tissue will rapidly depolarize the light and PS-OCT can be used to determine the degree of depolarization of the backscattered light [21]. The intensity of backscattered light, namely from the dental caries, is measured as a function of its axial position in the tissue (See Figure 4E and 4F).

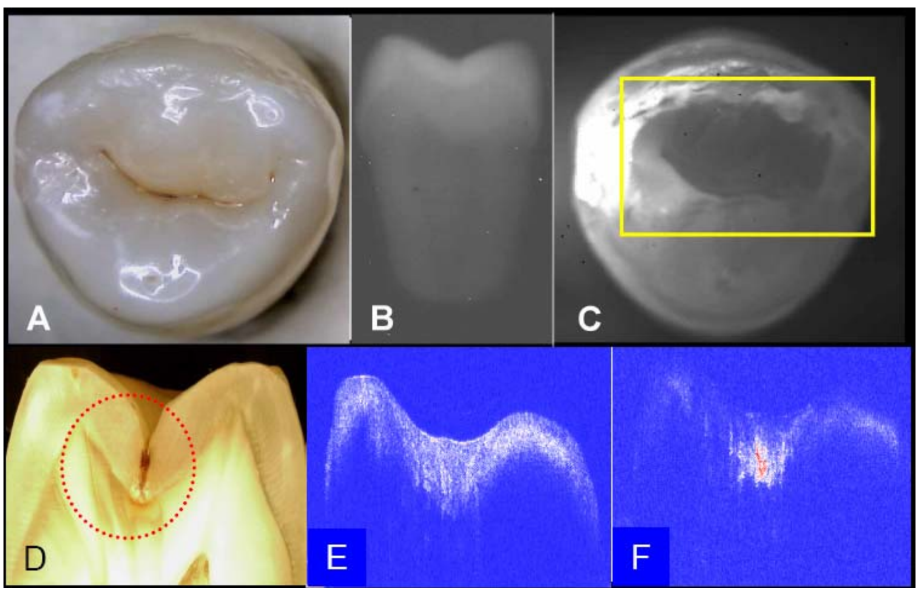


Figure 4. Premolar with a hidden subsurface lesion, the reflected light image (A) contains a deep, pigmented fissure, but it is not clear whether there is decay. (B) Radiograph (D-speed film) shows no decay. The NIR image (C) contains a large opacity in the yellow square. The tooth was cut in half along the dotted line in (C), and the tooth hemi-section (D) shows the lesion at the base of the pit (red circle). Note that there is no decay along the walls of the fissure. The decay does not show up very well in the PS-OCT parallel axis scan (E) taken transverse to the fissure while the PS-OCT perpendicular axis scan (F) clearly shows the decay at the base of the pit. Areas of high reflectivity are in red, moderate reflectivity in white, and low reflectivity in blue in the PS-OCT scans. (*From:* Buhler C, Ngaotheppitak P, Fried D. Imaging of occlusal dental caries (decay) with near-IR light at 1310-nm. Optics Express. 2005; 13(2): 573-582).

Optical Imaging of Dental Enamel

The principal limiting factor for optical imaging through the enamel of the tooth in the visible range (400-700nm) is light scattering. The scattering of light in the sound enamel and dentin is sufficiently strong in the visible range to obscure light transmission through the tooth. It is noted that with increasing wavelength, the light scattering in dental enamel decreases markedly. In fact, in the near-IR (1310nm) range, the scattering coefficient of enamel is approximately 2 to 3 cm^{-1} compared to 400 cm^{-1} in the visible range (400-700nm). However, at longer wavelengths, the attenuation increases due to water absorption [22-26] (Figure 5).

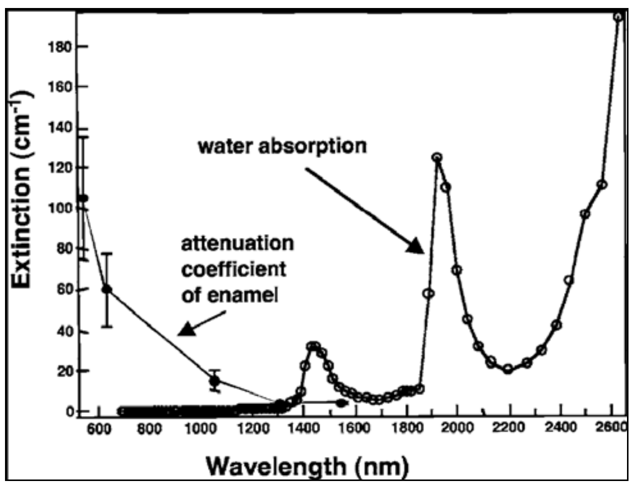


Figure 5. Plot of the extinction (attenuation) coefficient of dental enamel (filled circles) and the absorption coefficient of water (open circles) versus wavelength. (*From:* Darling CL, Huynh GD, Fried D. Light scattering properties of natural and artificially demineralized dental enamel at 1310nm. J Biomed Opt. 2006; 11(3).)

Therefore, the near-IR region at 1310nm offers the greatest potential for new optical imaging modalities due to the weak scattering and absorption in sound dental hard tissue. Dental caries, however, has a markedly high scattering coefficient. This is thought to occur as a result of an increase in porosity in areas of demineralization.

Increased backscatter from the demineralized region of early lesions is the basis for the visual appearance of white spot lesions. According to previous studies by Fried *et al.*, the optical scattering increases exponentially with mineral loss and the magnitude of light scattering approaches a maximum after 10-15% mineral loss that is consistent with the formation of pores. For higher volume percent mineral loss additional pores interconnect and the enamel falls apart forming a cavity above 40-50% mineral loss[22]. Image contrast between sound and demineralized enamel is at a maximum at 1310nm and an increase in light scattering upon demineralization provides a higher image contrast than that provided by variations in tissue density that occurs at x-ray wavelengths [25].

According to Ngaotheppitak *et al.* [19], since polarization sensitive optical coherence tomography measures changes in the magnitude of light scattering and the depolarization of scattered light due to demineralization, it has great potential for the diagnosis of the current “activity of the lesion” i.e. whether or not the caries lesion is active and expanding or whether the lesion has been arrested and is undergoing remineralization. It is noted that surfaces of arrested lesions are typically hard and shiny with less light scattering, in contrast to the soft and chalky surface of active lesions which scatter light orders of magnitude greater than arrested lesions. Therefore, PS-OCT is well-suited for the measurement of remineralization of early caries lesions, since it is capable of acquiring images of the subsurface structure of caries lesions. Stains, hypomineralization/fluorosis, pigmentation, and plaque do not interfere with the incident light signal and therefore, active caries lesions can be easily discriminated with NIR images at 1310nm.

Demineralization can be easily identified on PS-OCT scans to a depth of 2-3mm into the tooth. However, the location of the lesion will invariably change the contrast of the image. If the lesion is confined to the pits and fissures, it will have a sharp contrast due to the close proximity of the lesion to the surface. Deeper lesions are of larger width due to the spreading of the lesion along the DEJ and have low contrast since the bulk of the lesion is underneath the enamel. This latter point is important for investigations of PS-OCT on primary teeth because of their thin enamel and the rapid spread of caries through the enamel and dentin.

Imaging of Primary Teeth and Measurement of Scattering Properties

NIR imaging methods are ideally suited for imaging primary teeth. The advantages of this method is that exposure to ionizing radiation is not necessary and lesion progression may be followed with subsequent imaging without the harmful effects inherent with X-rays. Furthermore, a carious lesion can be imaged by positioning the camera on the occlusal and/or buccal/lingual surfaces of the tooth as shown in figure 6 to obtain a better location of the lesion. Deciduous teeth are also small and accessible and the clinician can avoid the difficult procedure of placing x-ray films in uncooperative children.

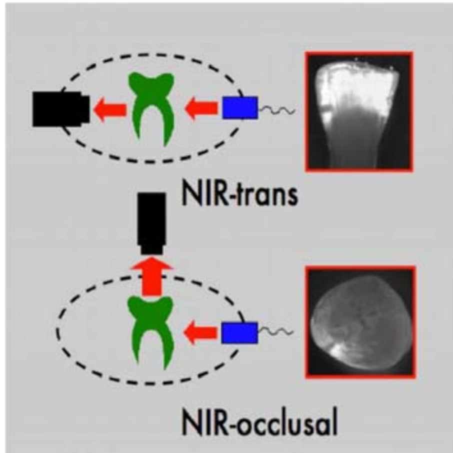


Figure 6. NIR imaging setup for NIR-trans (top) and NIR-occlusal (bottom) of tooth. (*From*: Hirasuna K, Fried D, Darling CL. Near-IR and PS-OCT imaging of developmental defects in dental enamel. J Biomed Opt. 2008; 13(4).)

Therefore, investigations in NIR imaging of deciduous teeth holds great potential in the management of caries disease in young children and adolescents. Caries progression/regression can be monitored on a regular basis and proper interventions can be implemented depending on the severity of the lesion. Dental caries is a preventable disease and with proper detection of early lesions and conservative, non-surgical therapy such as antimicrobial rinses, fluoride applications, and other preventative measures, the prevalence of the disease is expected to decrease. Therefore, the objective of this investigation will be achieved through the following specific aims.

AIM # 1: *To test the hypothesis that the scattering coefficient of sound deciduous enamel is orders of magnitude less at 1310 nm than in the visible range.*

Primary teeth have a different structure and composition than permanent teeth. Namely, deciduous teeth have a higher organic/mineral ratio compared to permanent teeth which is expected to influence its optical properties. Therefore, it will be necessary to measure the magnitude of the attenuation coefficient of the sound deciduous enamel at varying thickness at 1310 nm using Beer's law, eq.1, and a Beer-Lambert plot using the same methods used previously for permanent teeth (see reference 24).

$$\ln (I/I_o) = -\mu t \qquad (1)$$

AIM # 2: *To test the hypothesis that trans-illumination with NIR can discriminate carious enamel from sound enamel with high contrast.*

NIR images will be taken of deciduous enamel samples that have carious lesions confined to the enamel. The presence of these areas of demineralization will be confirmed by PS-OCT, polarized light microscopy (PLM), and with digital transverse microradiography (TMR). Contrast ratios will also be obtained.

AIM # 3: *To test the hypothesis that PS-OCT can image and identify caries lesions on deciduous teeth.*

PS-OCT images of deciduous teeth will be taken. The images will then be sorted and the presence of the lesion will be confirmed by comparing the PS-OCT image with images obtained from TMR and PLM techniques. We expect positive correlation of the location of the carious lesion using PS-OCT with the other imaging techniques.

Materials and Methods

Deciduous teeth (n=30) were collected from the pediatric dentistry clinic at the University of California, San Francisco with CHR approval. The teeth were sterilized using gamma radiation and stored in 0.1% thymol solution. Afterwards, the teeth were mounted on 1 x 1 x 3-cm^3 acrylic blocks with black orthodontic resin. The sides of the blocks were engraved with an ordinal ranking scale starting with "100" in order to keep track of the teeth. After properly labeling the teeth, photographs were taken from the occlusal and interproximal surfaces with a macro lens mounted on a Canon EOS 350D Rebel XL camera. The pictures were then uploaded to the computer for later analysis.

NIR_{trans} and NIR_{occ} of Selected Whole Teeth with Carious Lesions

The first experiment performed was the NIR imaging of the whole teeth. The NIR imaging setup is shown schematically in Figure 7. Both deciduous molars and incisors were selected for analysis. The optical imaging system used in this study is a Goodrich SUI™ KTS series InGaAs and Indigo NIR cameras each with a focal plane array of 318 x 252 pixels. Light from a single-mode fiber pigtail coupled to a 1310-nm superluminescent diode (SLD) with an output power of 15mW and a 35-nm bandwidth was coupled to a 20-mm NIR fiber collimator. Neutral density filters were used to attenuate the incident laser beam. The sample was mounted on a revolving post and images were taken from the occlusal surface at 30^0 intervals. Approximately 18 images per tooth were acquired. The same procedure was conducted to capture the interproximal

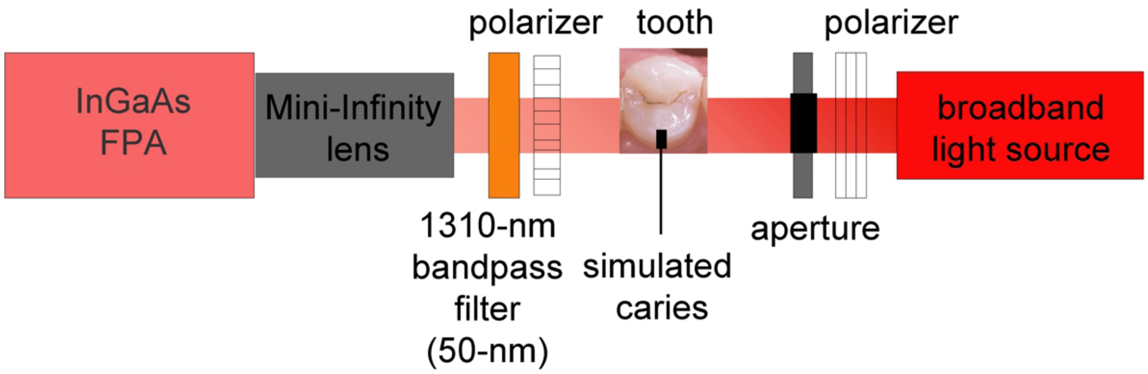


Fig. 7 Setup used for NIR Transillumination of whole teeth consists of a broadband light source, crossed linear polarizers, a bandpass filter and a NIR InGaAs focal plane array. (*From:* Jones RS, Huynh GD, Jones GC, Fried D. Near-infrared transillumination at 1310-nm for the imaging of early dental decay. Optical Society of America. 2003.)

surfaces of the deciduous teeth. Transillumination of the teeth was done at 20^0 intervals. All the acquired images were analyzed using IR Vista (Indigo Systems) software. Examples of NIR_{trans} and NIR_{occ} are shown in Fig 8.

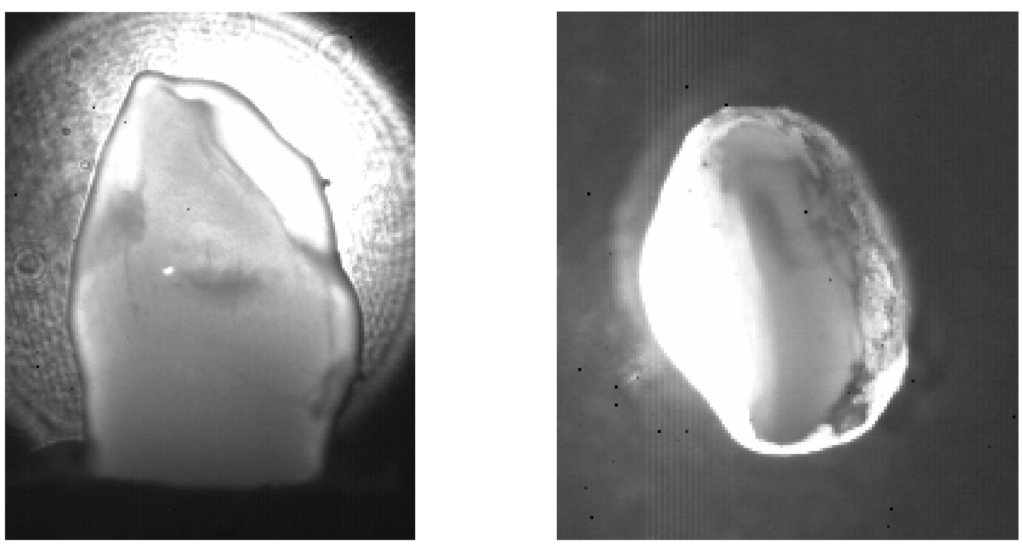


Figure 8. NIR_{trans} and NIR_{occ} images of a deciduous incisor

Image Analysis

Lesion contrast was measured for 12 samples as follows:

$$\text{Lesion contrast (C)} = (I_{sound} - I_{lesion}) / I_{sound} \quad (2)$$

where I_{sound} is the mean intensity of the sound enamel bordering the lesion and I_{lesion} is the mean intensity of the lesion. Lesion contrast is defined as a ratio that will vary between 0 to 1.

Polarization Sensitive - Optical Coherence Tomographic Imaging (PS-OCT)

Whole tooth samples containing suspected occlusal and interproximal lesions were selected by visual examination for imaging by PS-OCT. Teeth were brushed with 1% detergent solution and rinsed with doubly de-ionized water. The surface of the teeth showing natural caries lesions was not polished or modified in any way before images were acquired. A total of 30 extracted teeth were examined using PS-OCT (See Figure 9).

A single-mode fiber autocorrelator-based Optical Coherence Domain Reflectometer (OCDR), HSR-3000-P, custom designed and fabricated by Optiphase, Inc. (Van Nuys, CA) with a polarization switching probe, high efficiency piezoelectric fiber-stretchers and two balanced InGaAs receivers was used for this study. This OCDR was integrated with a broadband high power superluminescent diode (SLD) (Denselight, Jessup, MD) with a center wavelength of 1314 nm, an output power of 48-mW and a bandwidth of 33-nm.

A high-speed XY-scanning system, ESP 300 controller & 850-HS stages, Newport (Irvine, CA) was used for lateral movement of the tooth samples at the focus of the optical probe for *in vitro* optical tomography. A pair of Faraday rotators build into the probe assembly were used to switch the polarization with the sweep rate of 50-Hz. The system was configured to provide a lateral resolution of approximately 50-μm over a depth of focus of 10-mm and an axial resolution of 16-μm in dentin and cementum. The interferometric signal was electronically demodulated and filtered and processed using LabVIEW™ software (National Instrumentes, Austin, TX). PS-OCT images were post-processed with a 10 x 10 Median filter to reduce the effect of speckle noise using image processing software, Igor Pro (Wavemetrics, Lake Oswego, OR).

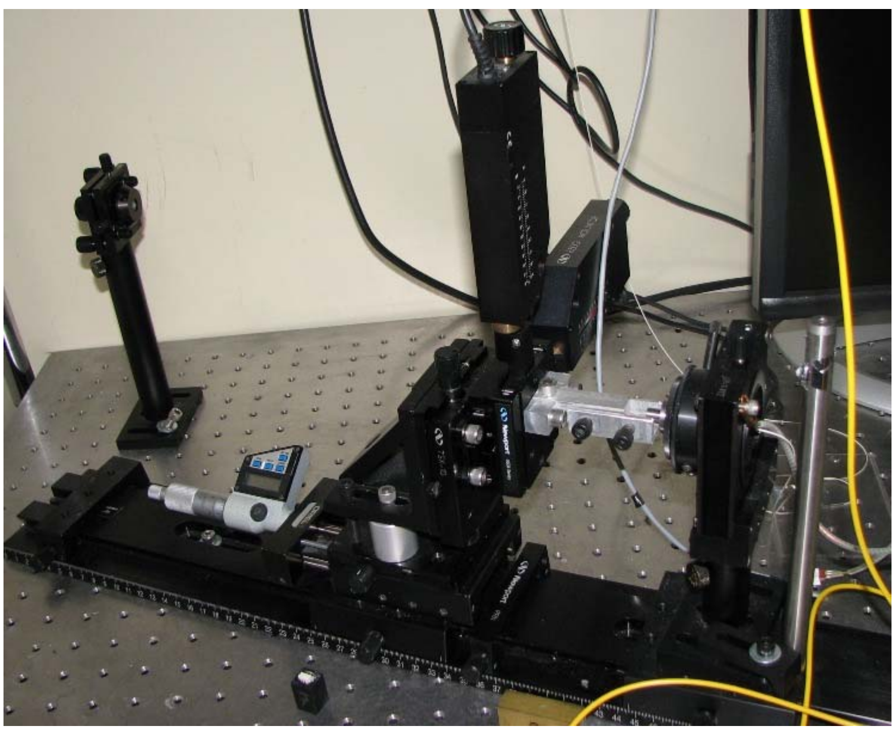
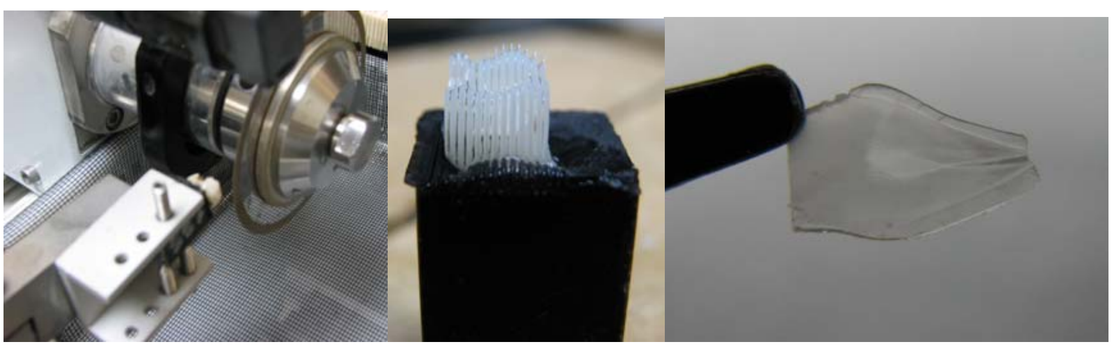


Figure 9. PS-OCT Setup

Sample Preparation and NIR imaging

After scanning, the samples were serial sectioned to produce matched thin section of 100 – 200 *μ*m thickness for NIR imaging, histological examination using polarized light (PLM) and mineral density determination using digital microradiography (TMR). The resin blocks fit precisely in a Buehler Isomet 5000 Linear Precision Saw (Buehler, Lake Bluff, IL) for cutting aligned thin sections. The rectangular geometry of the mount facilitates matching the position of plano-parallel OCT b-scans to the serial thin sections produced for TMR and PLM. Approximately 10 samples were obtained from each tooth. See Figure 10 below.



A **B** **C**

Figure 10. (A) Resin block with the embedded tooth being sectioned with the Buehler Isomet 5000 Linear Precision Saw, (B) Tooth after being sectioned, (C) Sample of ~150 μm thickness

From the collected samples, 15 were selected based on the presence of a carious lesion or white-spot demineralized region in the enamel. Finding samples with a lesion limited to the enamel proved difficult due to the relatively small thickness of enamel in deciduous teeth compared to the enamel thickness of permanent teeth. Most teeth obtained from the UCSF pediatric dental clinic were extracted from children with gross decay, often with caries approaching the pulp. Once these grossly decayed teeth were sectioned, very little enamel remained. The selected samples with smooth surface lesions were polished to a 5 μm finish.

The same optical imaging system that was used for the whole section was used to obtain images of the tooth sections. The samples were mounted on an acrylic post and inserted in a cuvette filled with deionized water. The acquired 8-bit digital images were analyzed using the Goodrich™ software. The setup is shown in Figure 11.

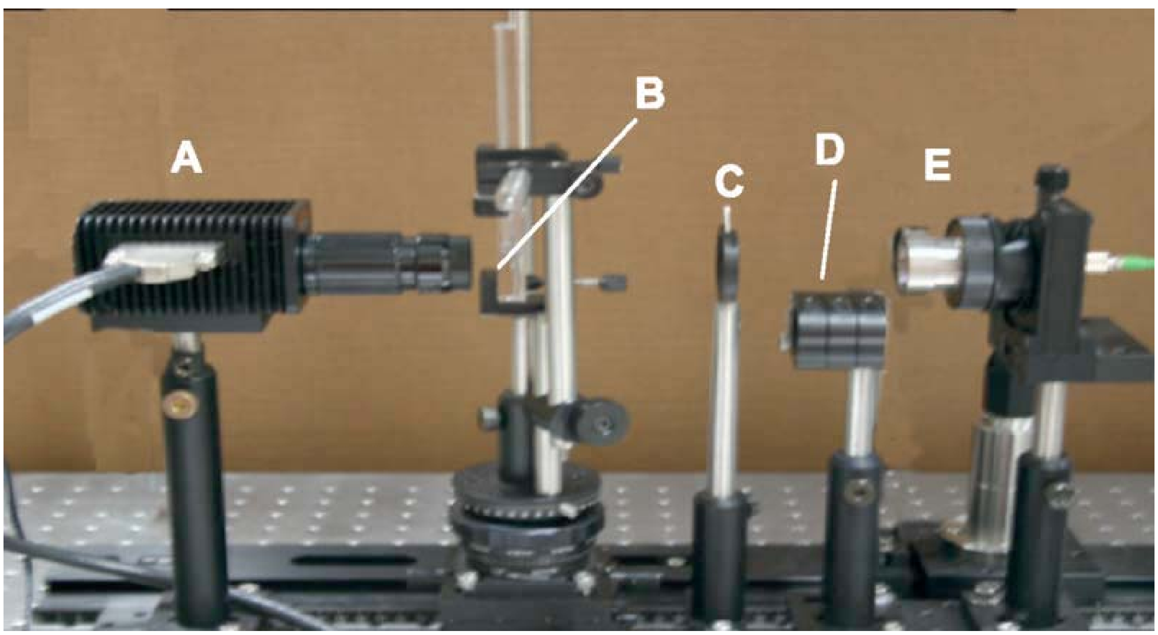


Figure 11. Near-IR imaging setup: (A) InGaAs FPA, (B) cuvette and section holder, (C) aperture, (D) neutral density filters, and (E) laser fiber pigtail and collimator

Digital Transverse Microradiography

Transverse microradiography (TMR) is the currently accepted gold standard for the measurement of mineral loss in natural and artificial caries lesions. Therefore, TMR is considered the most appropriate method for validation of new caries imaging methods such as optical coherence tomography, terahertz imaging and fluorescence.

TMR was used to measure mineral loss in caries lesions in deciduous enamel. A custom-built digital microradiography system was used to measure mineral loss in the natural caries lesions. High-resolution microradiographs were taken using Cu Kα radiation from a Philips 3100 x-ray generator and a Photonics Science FDI x-ray digital imager (Microphotonics, Allentown, Pennsylvania). The setup is shown in Figure 12. The x-ray digital imager consists of a 1392 x 1040-pixel interline charge-coupled device (CCD) directly bonded to a coherent micro-fiber optic coupler that transfers the light from an optimized gadolinium oxysulphide scintillator to the CCD sensor. Images can be acquired in real time at a frame rate of 10 fps. The image size is 2.99 x 2.24 mm with a pixel resolution of 2.15 μm. A high speed motion control system with Newport UTM150 and 850G stages and an EPS300 controller coupled to a video microscopy and laser targeting system was used for precise positioning of the tooth samples in the field of view of the imaging system. From this technique, the quantitative mineral loss were obtained. The images obtained were analyzed using Igor Pro™, data analysis software from Wavemetrics Inc (Lake Oswego, Oregon). The sections were then examined using a polarized light microscope to measure the overall lesion depth.

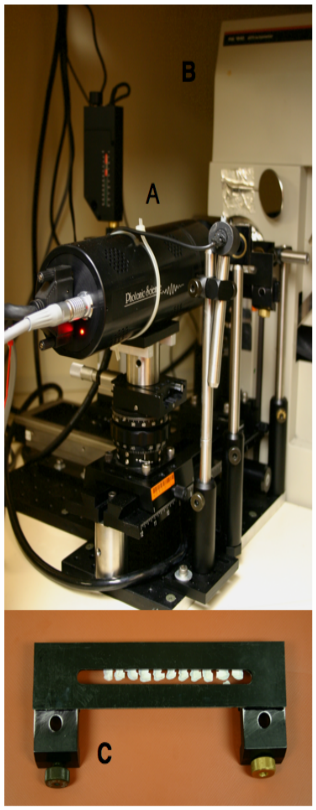


Figure 12. High-resolution digital X-ray microradiography system

Results

In this study, a total of 30 deciduous teeth were collected from the UCSF Pediatric Dentistry clinic. These teeth were sterilized with gamma radiation, mounted in acrylic blocks, photographed, and stored in a moist environment with 0.1% thymol solution to prevent fungal and bacterial growth. It is important to maintain hydration of the tooth to augment light penetration of the NIR and PS-OCT. There was no special preparation of the teeth before imaging.

All the teeth were imaged from the occlusal and interproximal surfaces with NIR light. Figure 13 shows a deciduous molar with a suspicious lesion in the mesial pit and distal groove.

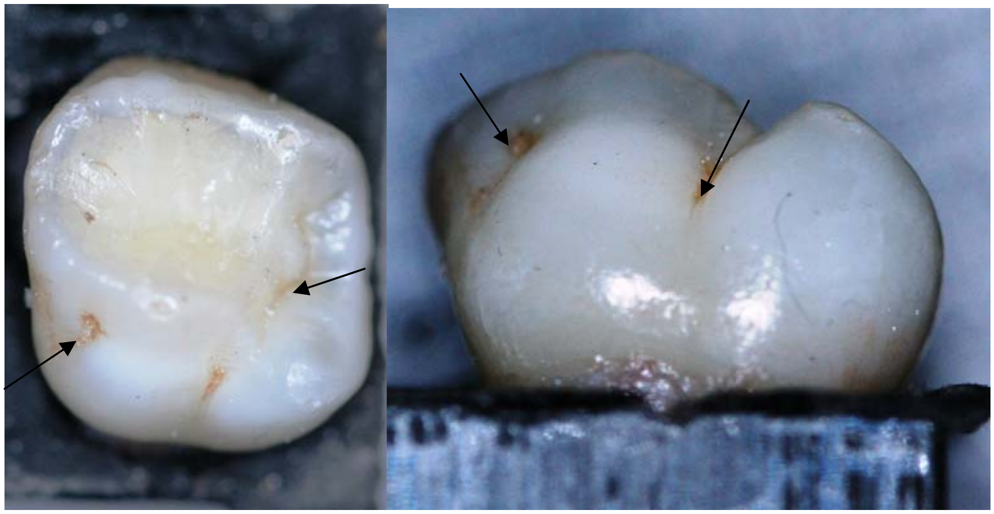
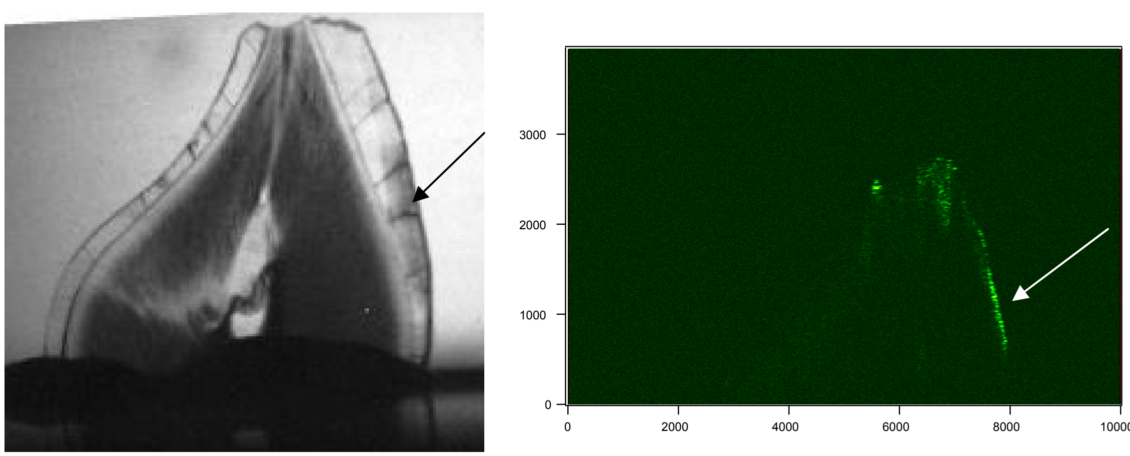


Figure 13. Occlusal and lingual views of a deciduous molar with a suspicious lesion in the distal groove and mesial pit. There is a 50% chance of correctly diagnosing this “lesion” as carious with conventional tactile, visual, and radiographic techniques.

The reflected light shows a dark pit at the center surrounded by brown areas. A dentist, as part of his/her routine examination, would use an explorer/visual approach to try to assess the status of the lesion. Furthermore, he/she may take an x-ray of the tooth and from the two methods, i.e. visual/tactile and x-ray, form a diagnosis. The problem with this approach is that it has a low sensitivity below 50% for detecting occlusal caries lesions. The same is true if an x-ray is taken in order to assess interproximal lesions. Often these lesions cannot be discerned due to overlap with the adjacent tooth or from radiographic anomalies such as cervical burnout. Moreover, radiographs may prove difficult when attempted on a young child in the pre-cooperative stage.

Another consideration is the presence of fluorosis (hypomineralization) on the deciduous tooth surface. Fluorosis, a developmental defect that appears as white blotches on the tooth surface, does not need to be treated and in the primary dentition, it is quite rare. According to Elfrink *et al.*[27], the prevalence of hypomineralized second primary molars is 3.6%. Unfortunately, early dental caries (demineralization) also appears white and cannot be easily discriminated from fluorosis. The NIR image, however, can discern between an area of demineralization (dark areas) and an area of fluorosis (white areas) in the NIR image. In fact, according to a recent study by Hirasuna *et al.*[28]," enamel defects [e.g. fluorosis] on whole teeth that could be imaged with high contrast with visible light were transparent in the NIR."

PS-OCT images were taken of the whole tooth samples. The images were compared to the images obtained with NIR in order to obtain a depth-resolved depiction of the lesion. Figure 14 shows a lesion confined in the enamel of deciduous teeth. A NIR image of the tooth confirms the presence of the lesion and the PS-OCT image shows the amount of demineralization as seen by the amount of light intensity.



A B

Figure 14. NIR (A) and PS-OCT image (B) taken of a deciduous incisor depicting a smooth surface lesion. The PS-OCT image shows the amount of demineralization of the lesion based on the light intensity of the sample, i.e. the greater the amount of demineralization, the greater the intensity of the light.

Lesion contrast measurements were taken of 12 representative whole tooth samples. Since many teeth that were used in this study had gross decay with large caries lesions, selection of the samples for lesion contrast measurements were based on image quality. Table 1 shows the various contrast ratios obtained using equation 2 for the 12 samples.

Table 1. Contrast Ratios

Sample	I_{sound}	I_{lesion}	Contrast ratio
100	202.49	155.45	**0.23**
102	163.14	143.69	**0.12**
107	144.98	122.94	**0.15**
109	125.92	111.13	**0.12**
111	156.12	104.68	**0.33**
113	150.25	111.62	**0.26**
117	140.28	119.48	**0.15**
121	158.09	100.47	**0.36**
122	144.58	84.90	**0.41**
125	124.24	78.22	**0.37**
126	145.94	110.53	**0.24**
127	185.10	128.34	**0.31**

The lesion contrast was calculated for the 12 samples using equation 2 (pg. 20). Contrast ratios have a range from 0 to 1, with 0 indicating no contrast between the lesion and sound enamel and 1 indicating that the lesion completely blocks light transmission. Contrast values above 0.2 are considered quite high. The mean contrast ratio in our study is 0.25 +/- 0.10.

These results differ slightly from a study by Jones RS *et al.*., in which a lesion contrast of greater than 0.35 was seen in thirty plano-parallel sections of permanent dental enamel ranging in thickness from 2 to 7.5mm[25]. Proper comparison with the results of this study is difficult since the samples used in this investigation were of whole deciduous teeth. However, Jones RS *et al.*. and a study by Jones GS *et al.*[26] demonstrate that there is a trend of the contrast ratio being inversely proportional to the sample thickness.

After imaging, the teeth were cut into slices ranging from 100 – 200 μm thickness. Using visual examination, samples with lesions/demineralized areas confined to the enamel were selected. The presence of lesions/demineralized areas was confirmed using polarized light microscopy (PLM). The use of PLM yields a high quality image of the lesion (Figure 15).

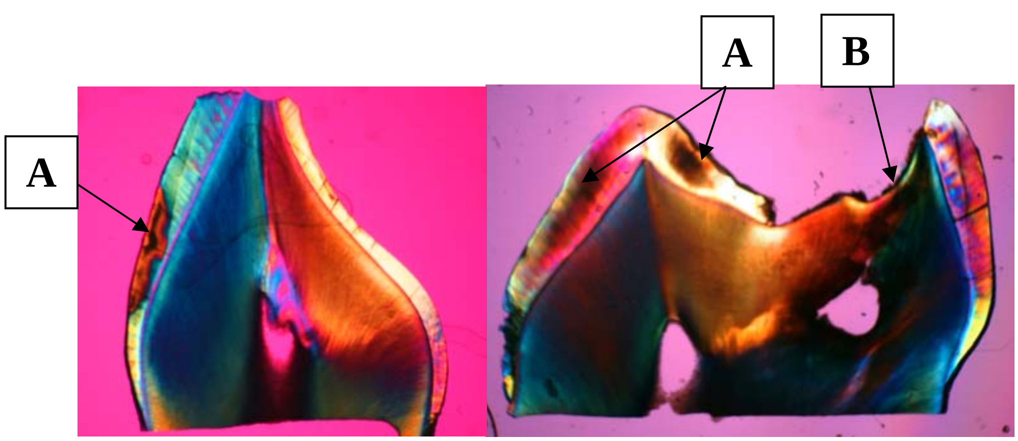


Figure 15. Images obtained using polarized light microscopy (PLM). (A) Presence of a caries lesion confined within the enamel on a deciduous incisor and molar. (B) lack of enamel due to extensive decay

A total of 15 samples was selected from a source of approximately 300 samples. These samples were polished to a 5 *μ*m finish. Polishing of the samples is important in order to remove any marks and imperfections that remained after the teeth were sectioned into thin slices. Furthermore, it improves the quality of the image obtained when imaging these samples with NIR light.

The images obtained with NIR light were compared to those obtained with PLM. Of the 15 samples, 10 were selected based on specific criteria for digital transverse microradiography (TMR) analysis. The criteria used for selecting the teeth included: sound enamel present on the sample and confinement of the lesion on the enamel surface (see figure 16).

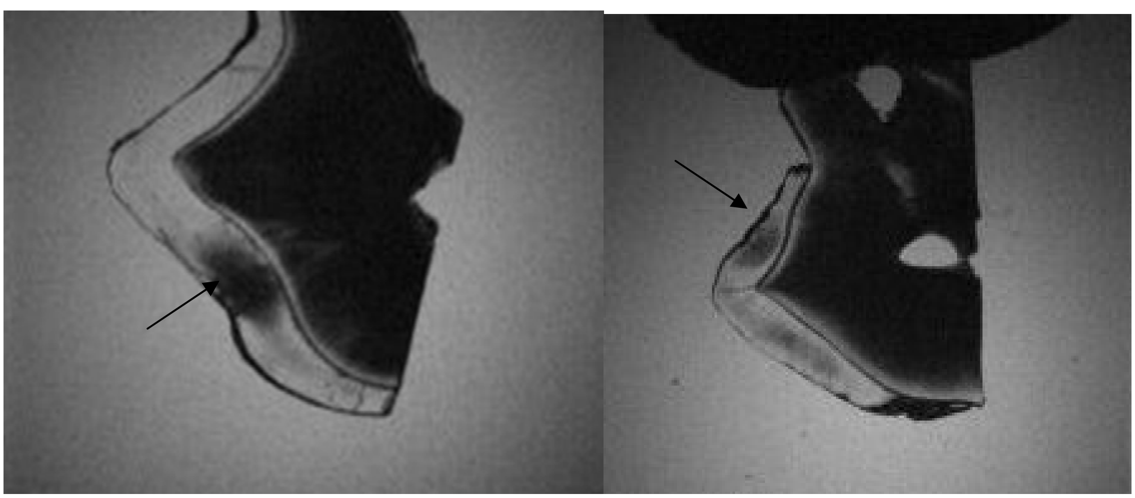


Figure 16. NIR image of samples from deciduous molars showing carious lesions (*see arrows)* confined to enamel.

Sound enamel is needed in order to properly assess the differences in mineral content of the sound enamel surface with the lesion(s). Furthermore, a lesion confined to the enamel surface is important due to the limitations of imaging past the enamel surface into the dentin with NIR light.

The first step in determining the optical properties of carious enamel was to establish a method of characterizing the severity of the carious lesion. The gold standard in cariology research is mineral loss, which defines the degree of demineralization in the lesion. The most severe lesions have lost a higher volume fraction or volume percent of their original mineral content than less severe lesions[22]. TMR analysis was used to measure mineral loss in the caries lesions of the ten (10) selected samples. TMR allows real-time acquisition of digital x-rays and works well for thin sections greater than 80 μm, since it is difficult to cut 80 μm thin sections without destroying natural lesions. The microradiograph of a lesion is shown in Figure 17. Figure 17 contains an image of the processed x-ray data that is presented as volume percent mineral—sound enamel is approximately 91% vol.% mineral. The line profile shown in the bottom of figure 17 was taken across the lesion and reflects the severity and topography of the lesion.

Similar NIR and TMR images were acquired from ten caries lesions. Although ten samples were selected for analysis, unfortunately one sample was lost due to iatrogenic fracture. Therefore, nine samples remained and a plot of the optical attenuation versus volume percent mineral loss for ninety data points (10 points per sample) was assembled from all the line-profiles across the most highly demineralized area of each lesion. The plot is shown in Figure 18. Nonlinear regression using an exponential growth curve shows that the optical

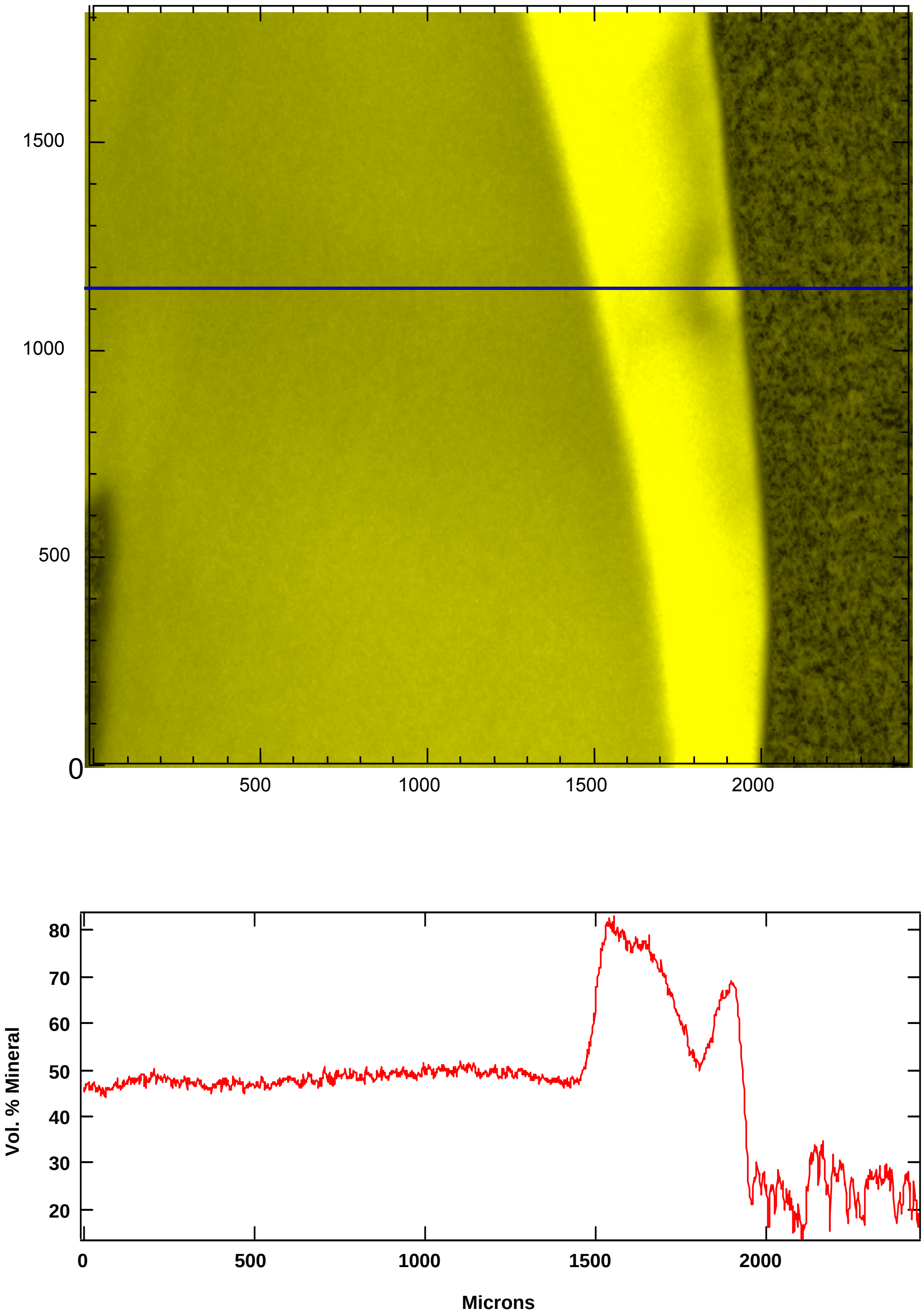


Figure 17. (top) A high-resolution digital microradiograph of the lesion area shows the volume percent mineral versus position in the lesions. (bottom) Volume percent mineral versus position for the horizontal line drawn through the lesion.

scattering increases exponentially with mineral loss and that the magnitude of light scattering approaches a maximum after approximately 15-20% mineral loss, which is consistent with the formation of pores. For higher volume percent loss, additional pores interconnect and the enamel falls apart, forming a cavity above 25-30% mineral loss.

The literature has shown that primary dental enamel has a 20- to 100-µm thick prismless or aprismatic zone at the surface. Within the prismless zone, all the crystals are aligned parallel to each other and perpendicular to the surface. The nature of this zone, however, may not be constant around any individual tooth. The prismless layer is generally more highly mineralized due to the parallel nature of the crystals and the lack of prism boundaries, and this layer is more acid resistant[29]. Therefore, comparing the plot of the optical attenuation versus volume percent mineral loss for *deciduous* teeth with the plot for *permanent* teeth (Figure 19), there is a "slow rise" of the line on the deciduous plot due to little optical scattering occurring until the volume percent mineral loss exceeds 10%. Presumably, this is due to the highly mineralized prismless layer that allows transmission of the NIR light with little or no scattering. It can be hypothesized that as greater demineralization occurs, the attenuation coefficient increases and plateaus at approximately 180. In contrast, the plot of the permanent tooth enamel demonstrates a high increase in the attenuation coefficient at lower levels (between 0 and 20% mineral loss) compared to deciduous tooth enamel, possibly due to the absence of an aprismatic surface layer.

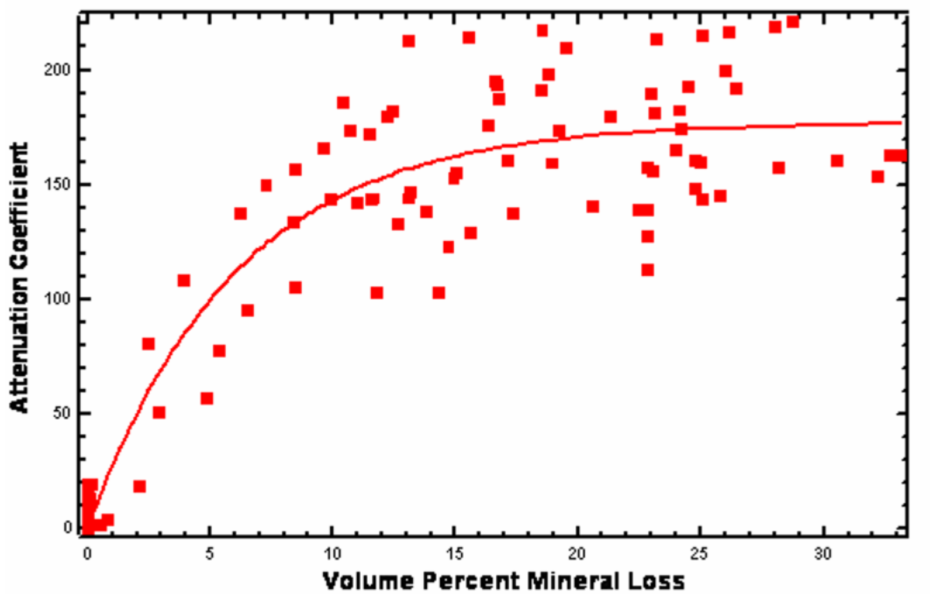


Figure 18. A plot of the optical attenuation versus volume percent mineral loss. 90 points taken from 9 natural deciduous enamel carious lesions (10 points per lesion). The solid line represents the best exponential fit, $r^2 = 0.83$ to the attenuation coefficient versus volume percent mineral loss.

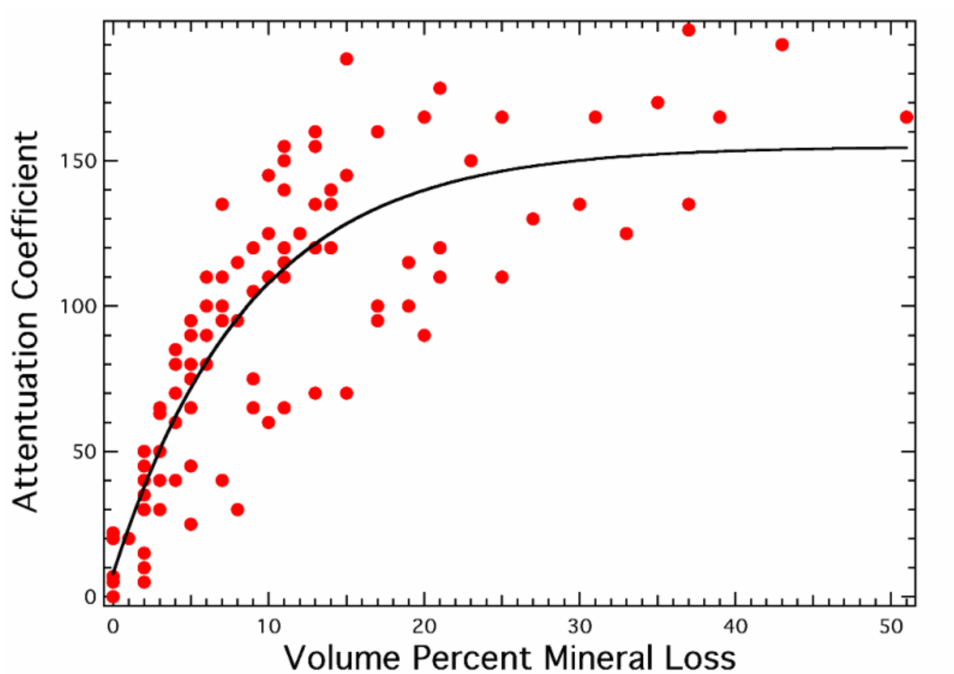


Figure 19. A plot of the optical attenuation versus volume percent mineral loss for permanent teeth. (*From*: Darling CL, Huynh GD, Fried D. Light scattering properties of natural and artificially demineralized dental enamel at 1310nm).

DISCUSSION

During the caries process, pores are formed in the lesion due to partial dissolution of the individual mineral crystals. Such small pores act as scattering centers, strongly scattering visible and near-IR light. Furthermore, the demineralization proceeds along the core of the enamel rods, hence the scattering centers may be fairly large in the micron range and highly anisotropic. This also suggests that many of the pores in the lesion are Mie-like scatterers with scattering centers of a similar dimension to the enamel prisms[22]. Such an observation is important for the interpretation of optical coherence tomographic images, since the lesion contrast is due to an increase in back-scattered light from the lesion area. Moreover, NIR imaging of occlusal and interproximal carious lesions exploits the high transparency of dental enamel and the strong scattering and weak absorption of the underlying dentin to deliver a uniform distribution of diffuse NIR light underneath the transparent enamel of the crowns to facilitate high contrast NIR imaging of the smooth surface lesion(s) and/or areas of demineralization. As shown by Buehler[17], "NIR technology can be used to acquire images of dental decay that is not detectable by conventional means either radiographically or by visual/tactile examination". This technology also facilitates imaging true areas of demineralization without interference from fluorosis (hypomineralization), pigmentation, and staining. Such confounding variables interfere significantly with visual diagnosis of dental decay and cause false positives in fluorescent caries detection methods such as QLF.

NIR technology is a promising diagnostic method for detection of early caries in young patients. While existing methods of diagnosis, e.g. conventional x-rays and visual/tactile, are acceptable tools for caries detection, the literature has extensively shown the need for alternative, reliable methods for caries detection. These traditional methods for identifying caries, especially in its early stages, have proven to be ineffective and unreliable. In a review of conventional methods of caries diagnosis, ten Cate[30] indicated that visual and tactile diagnosis of occlusal caries typically has a very low sensitivity of approximately 0.30, implying that only 20-48% of the caries present (usually into the dentin) are found. Radiographic assessment of carious lesions also exhibits a low sensitivity of approximately 0.59. Furthermore, due to the convoluted topography of the dental crown, many lesions that are detected radiographically have progressed beyond the point that conservative therapy can be rendered. Another limitation with routine radiographs, especially bitewings is that this examination may be difficult to perform in children. Not only are their mouths smaller, making it difficult for the film to be positioned, but in some cases there is less tolerance, greater anxiety, and more limited understanding. Taking successful bitewing radiographs demands good behavior-management techniques. Being able to obtain radiographs without hurting or upsetting the child is important for the child's future dental visits. An unsuccessful first experience increases the chance of future rejection of dental treatment. Therefore dentists often face the difficult task of placing and holding an intraoral radiographic film steady during exposure in a child whose behavior may hinder the acquisition of a diagnostic film[31].

NIR imaging has considerable potential as a tool for routine caries screening of the entire dentition. Since the optical properties of permanent and deciduous enamel are similar (as shown by the results of this study), *in-vivo* NIR imaging can be performed in children in their primary or mixed dentition to monitor lesion progression. A novel clinical imaging system is currently being developed. The bench-top setup of Figure 11 can be converted into a clinical imaging system by connecting a endoscope attachment to the InGaAs FPA with a 90^{o} mirror with a small hand held fiber-optic probe to place against the side of the tooth to deliver the NIR light. This system has the advantage that quick high-quality images of the deciduous teeth can be obtained without demanding that the child be fully cooperative during the acquisition of the images. Presumably, this method can be used in pre-school children where behavior and the use of routine x-rays is a concern. In fact, since NIR imaging does not use ionizing radiation, multiple NIR images can be acquired during subsequent visits to determine if the fluoride therapy, xylitol use, and other non-surgical preventative techniques are effective in halting the caries process. Moreover, using PS-OCT in tandem with NIR imaging to acquire specific depth resolved images of the lesion depth and severity in suspect areas defined in the near-IR image and both these NIR imaging systems can potentially share similar broadband light sources. In fact, using both diagnostic methods together can presumably confirm the presence of the lesion (using NIR) and better establish its specific location and severity (using PS-OCT). Therefore, this technology can be used effectively in identifying early lesions in deciduous teeth, monitor the activity of a lesion in a child, and safeguard the patient against the harmful effects of repeated exposure to ionizing radiation.

One limitation with this study was that the majority of the samples collected originated from children whose primary teeth needed extraction due to extensive decay and infection. The teeth used had large cavitations and it was difficult obtaining samples with early lesions confined to the enamel. Therefore, further *in-vivo* studies are needed in order to assess the efficacy of a clinical NIR model with pediatric dental patients and to test how well this imaging technique is at monitoring lesions after preventative measures have been taken based on the child's caries risk.

In conclusion, the results of this study have confirmed the three initially postulated hypotheses. As discussed in this paper, NIR imaging at 1310nm is an effective method of discerning carious enamel from sound enamel with a high degree of contrast. Furthermore, the optical attenuation versus volume percent mineral loss plot for deciduous dental enamel shows unique properties but also demonstrates similar trends with permanent tooth enamel. The former can be attributed to the surface layer of enamel which presumably allows transmission of the NIR light with little or no scattering. Looking at the NIR images, the carious lesion is easily identified. By taking a PS-OCT scan, a depth-resolved image is obtained which indicates the severity of the lesion. From a clinical perspective, it would be useful to use PS-OCT in conjunction with NIR imaging to confirm not only the presence of an early carious lesion but also to assess the severity of the lesion based on the amount of demineralization present in the lesion.

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